

## TAXONOMY AND EVOLUTION OF *JUNIPERUS COMMUNIS*: INSIGHT FROM DNA SEQUENCING AND SNPs

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### ABSTRACT

Plants of *Juniperus communis* var. *communis* and var. *charlottensis*, var. *depressa*, var. *hemispherica*, var. *jackii*, var. *saxatilis* as well as *J. rigida* and *J. grandis* (outgroup) were sampled and SNPs from nrDNA, petN-psbM, trnD-trnT and trnS-trnG were examined. Several varieties were found to be very distinct: v. *jackii*, v. *hemispherica*, v. *oblonga*, and v. *charlottensis* with v. *jackii* as distinct from *J. communis* as from *J. rigida*. Sequence data support the colonization of *J. communis* from ancestral populations of the species in Asia, most likely using the Bering Land Bridge as opposed to long distance dispersal. Our results suggest that, potentially, two independent colonizations of the New World plus a secondary reverse movement from the New World to Kamchatka has taken place. *Phytologia* 93(2): 185-197 (August 1, 2011).

**KEY WORDS:** *Juniperus communis*, var. *charlottensis*, var. *depressa*, var. *hemispherica*, var. *jackii*, var. *megistocarpa* and var. *saxatilis*, Cupressaceae, geographic variation, nrDNA, petN, trnDT, trnSG, SNPs, Pleistocene migrations.

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*Juniperus communis* is the only species of *Juniperus* that occurs in both the eastern and western hemispheres (Adams, 2011). Analysis of Arctic populations of *J. communis* (Adams et al., 2003) revealed that these populations clustered by continent, with the populations in Greenland and Iceland showing the highest affinities to populations from Europe and not to those from North America (Fig. 1). Adams et al. (2003) concluded that the post-Pleistocene populations on Greenland

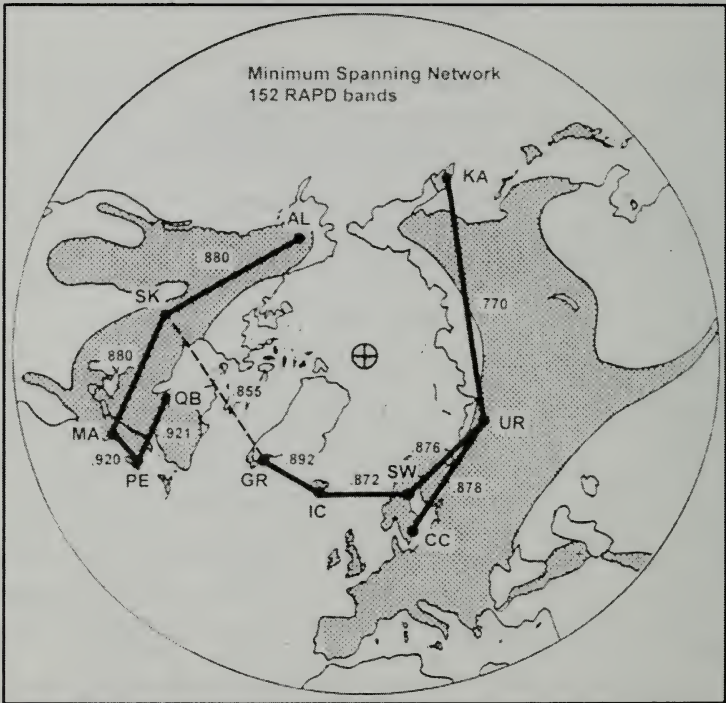


Figure 1. Minimum spanning network showing that the North American *J. communis* var. *depressa* and var. *megistocarpa* populations link together and all the *J. communis* populations from the e. hemisphere link together (Adams et al., 2003). The dashed line is the minimum link between eastern and western hemisphere populations. Shaded area shows the distribution of *J. communis*.

and Iceland came from Europe and not North America. Adams and Pandey (2003) analyzed *J. communis* and its varieties by use of RAPDs and found considerable variation, but several of the varieties were not discernable.

Adams and Nguyen (2007) collected additional samples of putative *J. c. var. saxatilis* from the Pacific northwest, *J. c. var. jackii* from NW California and *J. c. var. depressa* from the southernmost locations in North America (Mt. Charleston, Nevada and Mt. Satula, North Carolina). They found the major trend among the taxa was the separation of the eastern hemisphere plants (*J. communis* var. *communis*, *J. c. var. saxatilis*, and putative *J. c. var. saxatilis*, Kamchatka) from the western hemisphere plants (*J. c. var. depressa*, *J. c. var. jackii*, *J. c. var. megistocarpa*, and putative var. *saxatilis*). The resolution of *J. c. var. jackii* (and plants from Mt. Hood) was in contrast to the report by Ashworth, et al. (1999, 2001).

More recently, Adams (2008) examined nrDNA SNPs in varieties of *J. communis* in North America and found *J. c. var. jackii* to be very distinct (Fig. 2) along with the juniper from Queen Charlotte Island (recognized as *J. c. var. charlottensis* R. P. Adams). Interestingly, *J. c. var. depressa* and *J. c. var. saxatilis* (Japan) were found to be identical in their nrDNA.

The purpose of this paper is to report on the taxonomy and evolution of *J. communis* based on a more comprehensive taxon and data sampling using all varieties of *J. communis* and nrDNA, petN-psbM, trnD-trnT and trnS-trnG sequence data.

## MATERIALS AND METHODS

Specimens used in the present study: *J. communis* var. *communis*: Adams 7846-7847, Sweden; *J. c. var. charlottensis*: Adams 10304-10308, Queen Charlotte Island, BC, Canada; *J. c. var. depressa*: Adams 7802-7804, Victor, CO, USA; *J. c. var. hemispherica*: Adams 9045-9046, Mt. Etna, Sicily; Adams 7589-7590, Sierra Nevada, Spain; *J. c. var. jackii*: Adams 10287-10291, serpentine, Del Norte Co., CA; USA; *J. c. var. megistocarpa*: Adams 8575-8576, Magdalen Island, Quebec, Canada; *J. c. var. nipponica*: Adams 8579, 8690, Japan (ex Jin

Murata); *J. c.* var. *oblonga*: Adams 8764-8765, Lake Sevan, Armenia; *J. c.* var. *saxatilis*: Altair Mtns., Mongolia; Adams 7194-7195, Adams

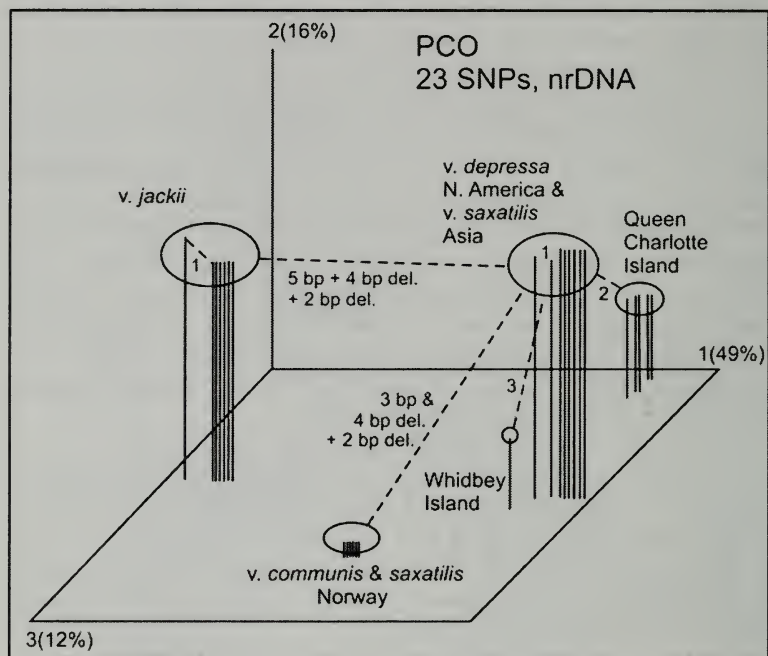


Figure 2. PCO of 58 individuals based on 23 SNPs. Identical bars, closely spaced indicate no variation among these individuals. The bars in the largest group are symbolic, as that group contains 27 individuals!

11206, 11207 Norway; Adams 10890-10893, Redfish Lake, Idaho; Adams 9181-9183 Esso, Kamchatka, Peninsula, Russia (ex. J. W. Leverenz); Adams 8686-8687, Hokkaido, Japan (ex Naotoshi Yoshida); Adams 11088, 11090, Sakhalin Island, Russia (ex Hideki Takahashi 31164, 31094); Adams 10185-10186, Urup Island, Kurils, Japan (ex M Ohara sn, Hideki Takahashi 22211); *J. rigida*: Adams 8544-8545, (ex Jin Murata), Honshu Island, Japan; *J. grandis*: Adams 11963-11964, Meyers, CA. Voucher specimens are deposited at the Baylor University herbarium (BAYLU).

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at  $-20^{\circ}\text{C}$  until the DNA was extracted. DNA was extracted using the Qiagen DNeasy mini kit (Qiagen Inc., Valencia CA).

**PCR amplification:** Amplifications were performed in 30  $\mu\text{l}$  reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15  $\mu\text{l}$  2x buffer E (petN, trnD-T, trnL-F, trnS-G) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200  $\mu\text{M}$  each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM  $\text{MgCl}_2$  according to the buffer used) 1.8  $\mu\text{M}$  each primer. See Adams and Schwarzbach (2011) primers utilized. The primers for nrDNA, petN-psbM, trnD-trnT, and trnS-trnG regions have been previously reported (Adams and Kauffmann, 2010). See Adams and Schwarzbach (2011) for band purification and sequencing procedures as well as data analysis.

## RESULTS AND DISCUSSION

Concatenation of nrDNA, petN-psbM, trnD-trnT and trnS-trnG sequences resulted in 3603 bp of data. A NJ tree (Fig. 3) shows several surprising results. *Juniperus communis* var. *jackii* (nw USA) grouped outside *J. communis* and its varieties, with *J. rigida* being more closely related to the other *J. communis* varieties (Fig. 3). *Juniperus c.* var. *jackii* has a very unusual habitat (serpentine and volcanic lava), morphology and leaf oils. Adams et al. (2010) reported that var. *jackii* leaf essential oil is low in  $\alpha$ -pinene, high in  $\delta$ -3-carene, and terpinolene (as found in *J. c.* var. *saxatilis* from Switzerland). In addition, var. *jackii* has 6 diterpenoids that are not found in any other *J. communis* variety, except in *J. c.* var. *saxatilis* from Switzerland (Adams et al. 2010). It is not surprising that it is distinct from other North American members of *J. communis*, but to be as distinct as *J. rigida* is surprising.

In contrast, *Juniperus communis* var. *megistocarpa* is not resolved as a distinct clade. Yet, it has the most distinctively large female cones in the species and an unusual habitat, occurring on sand dunes on islands and seashores and a somewhat unusual leaf essential oil composition (low in  $\delta$ -3-carene and  $\beta$ -phellandrene, very high in limonene, no  $\alpha$ -terpinyl formate or germacrene B, and the presence of





Nevada, Spain (Fig. 3). The shrubs from the Kamchatka Peninsula, Russia are also in a well supported clade as are the putative var. *saxatilis* shrubs from Sakhalin Island (Fig. 3). Taxa referred to as var. *saxatilis* are found in three clades: with European-Asian *J. communis*; with North American *communis* and with Japanese taxa. *Juniperus c. var. saxatilis* was described from Russia and the name is commonly applied to shrubs having leaves with the stomatal band 2-3x as wide as the green side bands (Adams, 2011). Although putative var. *saxatilis* from North America and Japan satisfy those morphological characteristics, it appears (Fig. 3) that three taxa are present and var. *saxatilis (sensu stricto)* only occurs in Europe and central Asia.

The Bayesian Tree (Fig. 4) is very similar to the NJ tree (Fig. 3). Additional trees (parsimony and maximum likelihood) gave similar, but less resolved trees. As the *communis* varieties are likely freely hybridizing and are of very recent origin (Mao et al. 2010), tree building programs may not be appropriate for these kinds of data.

The NJ tree does not take into account indels and indels are very common in the cpDNA regions sequences. To assess the total mutations, indels were coded. Analysis of the 3603 bp data set revealed 135 mutational events (nucleotide differences plus indels). The nucleotide polymorphisms (ignoring single events among the accessions) plus indels are noted as SNPs (although technically SNPs refers to nucleotide differences). A minimum spanning network shows (Fig. 5) the major trend to be the tremendous differentiation of var. *jackii* (19 SNPs!) as well as a closely related species, *J. rigida*, by 20 SNPs.

As previously mentioned, var. *jackii* has a number of differences from other *communis* taxa. *Juniperus c. var. communis* (Sweden), the Norway shrubs (locally called *J. c. var. saxatilis*) and the Mongolian var. *saxatilis* shrubs are separated from other populations by 5 to 7 SNPs. The Mongolia shrubs are scarcely distinct from var. *communis* (2 SNPs, Fig. 5). The very long leafed plants from Armenia differed by 5 SNPs, lending support for the recognition of var. *oblonga*.

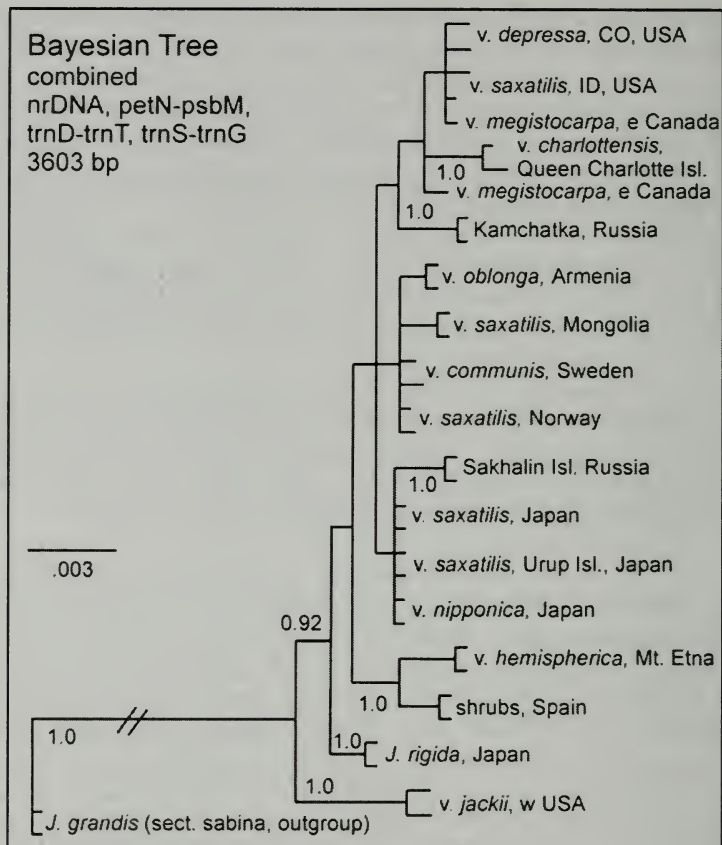


Figure 4. Bayesian tree based on 3603 bp of sequence data. Only those posterior probabilities greater than 0.90 are shown.

Adams and Pandey (2003) examined RAPDs from *J. c.* var. *hemispherica* from the type locality (Mt. Etna) and found them to be very similar to typical *J. c.* var. *communis* (Sweden). Based chiefly on these data, Adams (2011) treated var. *hemispherica* as a synonym of *J. c.* var. *communis*. The present sequence data gives support for the recognition of var. *hemispherica*, with its 7 SNPs differences from the shrubs in the Sierra Nevada, Spain and 11 SNPs differences from *J. c.*



var. *communis* (Fig. 5). Of considerable interest are the unusual shrubs (collected as var. *saxatilis*) from the Sierra Nevada, Spain. These differ by 9 SNPs from *J. c.* var. *communis* and 7 SNPs from var. *hemispherica* (Fig. 5).

The putative var. *saxatilis* (glaucous stomatal band 2-3x as wide as green side bands) from the western United States (Idaho) differs by 2 SNPs from *J. c.* var. *depressa/megistocarpa* (Fig. 5) and is not as closely related to typical var. *saxatilis* from the eastern hemisphere. In addition, the Kamchatka shrubs (called var. *saxatilis* by Adams 2008, 2011) appear to be more related to var. *depressa/megistocarpa* than to *J. c.* var. *saxatilis* from Europe and Asia (Fig. 5).

To examine the geographical variation in *J. communis*, a minimum spanning network was plotted onto the distribution map (Fig. 6.). In general, there appears to be considerably less variation in the western than in the eastern hemisphere. This may reflect the more recent colonization of the western hemisphere by *J. communis* (Mao et al. 2010).

A second trend is that the linkage of the western hemisphere to the eastern hemisphere by the Bering Land Bridge (BLB) seems stronger than across the Atlantic (Fig. 6). Mao et al. (2010) argued that *J. communis* could have come to the western hemisphere by land bridges or by long distance dispersal by birds (see Adams and Thornburg, 2010 for a review of seed dispersal in *Juniperus*). The present data give some support that *J. communis* came to the western hemisphere by the BLB.

The Kamchatka population is more closely related to North America plants than to nearby populations on the Urup and Sakhalin Islands (Fig. 6); This may reflect recent secondary migration from Alaska to Kamchatka during one of the several land bridges during the Pleistocene.

The divergence of var. *hemispherica* from Mt. Etna and var. *communis* from Sierra Nevada, Spain is rather large (Fig. 6). Additional research is being conducted to determine the extent of the divergence of Spanish *J. communis* in that region.

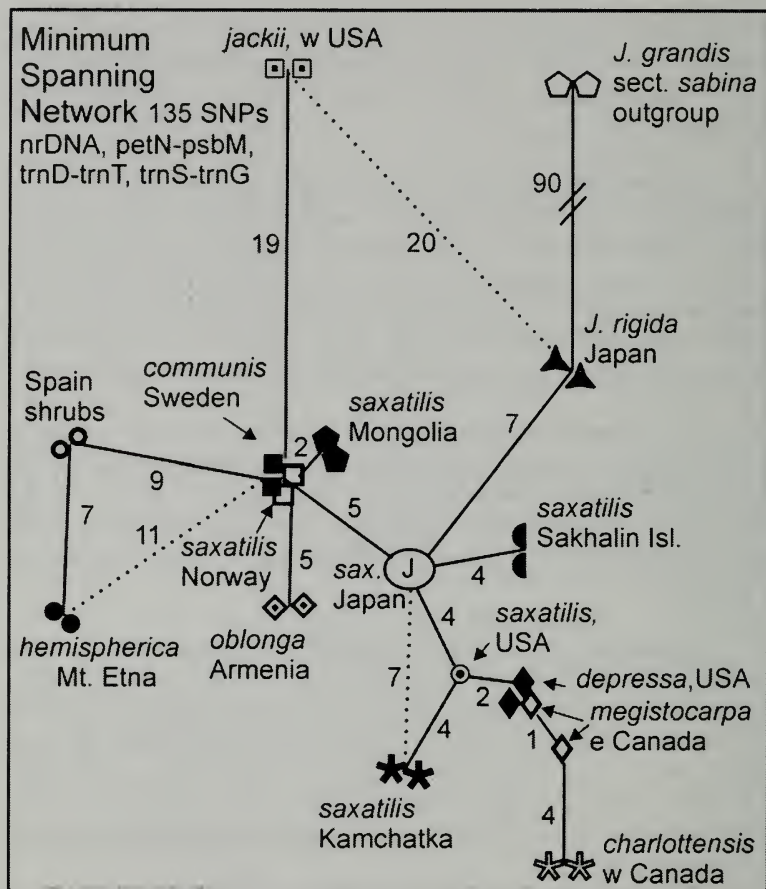
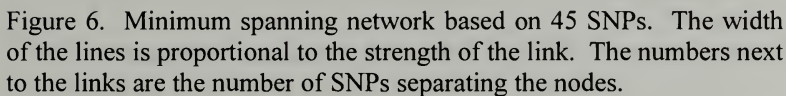


Figure 5. Minimum spanning network. The numbers next to the lines are the number of mutational events. The dotted lines are the second nearest link.

The distinct nature of *J. c.* var. *jackii* is shown in that its nearest links are 19 SNPs removed from *J. communis* (Sweden) and *J. c.* var. *charlottensis* (w Japan). Recall that var. *jackii* is 20 SNPs removed from *J. rigida* (Japan). Although var. *jackii* appears to be part



of the *communis* - *rigida* group, it is quite distinct in the sequences analyzed in this study. It seems possible that var. *jackii* (or its ancestor) may have been the first taxon of section *Juniperus* to migrate to North America. The *J. communis* var. *depressa* complex in North America is not very variable and appears closely related to *J. communis* from Japan. This complex appears to be a rather recent migration (Pleistocene?). Additional studies (in progress) are needed to elucidate the phyletic past of var. *jackii*.

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